

Susceptibility of Ripe Avocado to Invasive Alien Fruit Flies (Tephritidae) on the Island of Hawaii

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Abstract. The Avocado Growers Association on the island of Hawaii requested that the USDA-APHIS reconsider the possibility of approving the export of untreated avocado to the continental USA. In response, as part of the Hawaii Area Wide Pest Management Program, the Agricultural Research Service undertook a survey to supplement the original survey conducted by Liquido et al. (1995). This consisted of deploying traps baited with male lures for the three invasive species (*Bactrocera dorsalis* (Hendel), *B. cucurbitae* (Coquillett), and *Ceratitis capitata* (Wiedemann)) and protein bait traps for general detection of females within orchards. The survey was concentrated in the Kona District, and the orchards were mapped using a geographic information systems approach. In addition, between 9 August 2006 until 22 May 2007, 519 avocado fruits were collected from the ground and held individually to determine the presence of fruit fly larvae. Because male lure trap captures varied with locality and season and attracted flies from large distances, they are probably of limited value in predicting numbers of fruit flies within small avocado orchards. On the other hand protein bait traps, because they captured females and attracted flies from short distances, were a better indication of female flies found within orchards. *C. capitata* was the most prevalent species year round (0.456 ± 0.130 ♀ flies/trap/day). *B. dorsalis* was captured considerably less frequently (0.096 ± 0.068 ♀ flies/trap/day). *B. cucurbitae* was the least prevalent species in avocado orchards, averaging 0.034 ± 0.006 ♀ flies/trap/day. Adult fly emergence from the ground fruit sample was $1.25 \times 10^{-05} \pm 8.89 \times 10^{-6}$ *B. dorsalis* flies/g of fruit and no *C. capitata* emerged from any fruit sample. That is a fruit infestation rate of 0.385% and a rate of 0.771% larvae per fruit. All of the fruits sampled had some damage that would have excluded them from shipment by previous export criteria.

Key words: *Ceratitis capitata*, *Bactrocera* spp., *Persea americana*, quarantine

Introduction

The states of California and Florida have, for a long time, been concerned about the introduction of tephritid fruit flies, particularly Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). Since *C. capitata* has been present in Hawaii since 1907, fruit fly host fruit species are not allowed to be shipped from Hawaii to destinations within the continental USA. Nevertheless, tests conducted by the USDA-ARS (Agricultural Research Service) between 1983 and 1989 indicated that the avocado (*Persea americana* Mill.) cv. Sharwil was resistant to *C. capitata* infestation. On that basis, and with subsequent confirmation of that resistance (Armstrong 1991), the USDA-APHIS authorized the shipment of untreated 'Sharwil' avocados to the contiguous USA provided proper protocols were

observed (USDA-APHIS 1990). However, in 1992, routine inspections of packing houses by quarantine officials revealed 'Sharwil' fruit infested by *Bactrocera dorsalis* (Hendel), the oriental fruit fly. This led to a temporary ban on shipment of untreated avocado from Hawaii (USDA-APHIS 1992). Liquido et al. (1995) confirmed in a series of experiments in 1992-1993 that *B. dorsalis* could infest 'Sharwil' avocado under some conditions, and consequently a ban on export to the continental USA has been in effect ever since.

The avocado growers of Hawaii consider this ban an impediment to successful growth of their industry, particularly because untreated 'Hass' avocado are allowed to be shipped into the United States (including Hawaii) from Mexico. Data of Aluja et al. (2004) and Aluja et al. (2008) demonstrated that 'Haas' avocado was not infested by fruit flies of the genus *Anastrepha* (Schiner) and subsequent regulations (USDA-APHIS 1990, 2001, 2003) secured permission from USDA-APHIS to import 'Hass' avocado from Mexico, provided that the crop was grown under a systems management approach (Aluja and Liedo 1986). In Hawaii avocado production increased 25% between 2001 and 2005, but avocado imports doubled during the same period of time. This represents a 21.0% decline in Hawaii's market share (Anonymous 2005). 'Hass' avocado is popular with commercial chefs, and increased imports have put pressure on the Hawaii market. Subsequently, the avocado growers have repeatedly petitioned the Hawaii Dept. of Agriculture (HDOA) to encourage USDA-APHIS to lift the ban on export of Hawaiian 'Sharwil' avocados to the contiguous United States. For that purpose the USDA-ARS, at the request of USDA-APHIS and the HDOA, undertook another survey of the potential hazard of infestation of avocado by tephritid fruit flies. The objective of this study was to document the prevalence of tephritid flies in avocado orchards in the North and South Kona Districts of the Island of Hawaii, and to quantify the frequency of infestation of the most susceptible stages of avocado fruit development. This information may be useful in developing a systems approach to protecting 'Sharwil' avocados from fruit fly damage by possibly implementing the key area-wide pest management components 1) sanitation, 2) protein bait spray and 3) male annihilation (Vargas et al. 2008; Mau et al. 2007).

Materials and Methods

Trapping data. Monitoring began in the Kona District at 5 farms on June 1, 2006. There were five sites at one large avocado orchard in the South Kona District, and one site at each of the other small avocado orchards in the North and South Kona Districts. Each site consisted of 4 traps with lures: (1) methyl eugenol cones (10 g active ingredient [a. i.] methyl eugenol [4-allyl-1,2-dimethoxybenzene]) (Scentry, Billings, MT) for *B. dorsalis* males, (2) cuelure plugs (2 g a. i. cuelure {4-[p-Acetoxyphenyl]-2-butanone}) (Scentry, Billings, MT) for *B. cucurbitae* males and (3) trimedlure plugs (2 g a. i. trimedlure [tert-Butyl cis 4-chloro-trans-2-methylcyclohexanecarboxylate]) (FarmaTech, North Bend, WA) for *C. capitata* males). These solid lures and a Vaportape II (Hercon Environmental, Emigsville PA) DDVP toxicant strip, were placed inside a 1 liter polyethylene buckets (408-30N, Highland Plastics, Mira Loma, CA) or 5 liter polyethylene buckets (LT-804-165 Highland Plastics, Mira Loma, CA). The dimensions of the 1 liter bucket were 11.43 cm high, 11.90 cm in dia. and 36 g in weight, whereas the 5 liter bucket's dimensions were 20.32 cm high, 20.96 cm dia., and 148.5 g in weight. For both traps a 0.48 cm hole was drilled in the middle of the trap's lid. A 20 gauge insulated copper wire was cut to ca. 40.64 cm with 12.70 cm of insulation stripped from the bottom. The wire was inserted into the hole on the trap lid, bent where the insulation begins, glued, and then bent again to form a hook by which the traps were suspended in the canopy of avocado trees. The fourth trap, a yellow-bottom dome trap (Great Lakes IPM, Inc., Vestaburg, MI) containing protein bait in water was deployed at every site to monitor the female population. The protein bait was 8% Solulys (Roquette

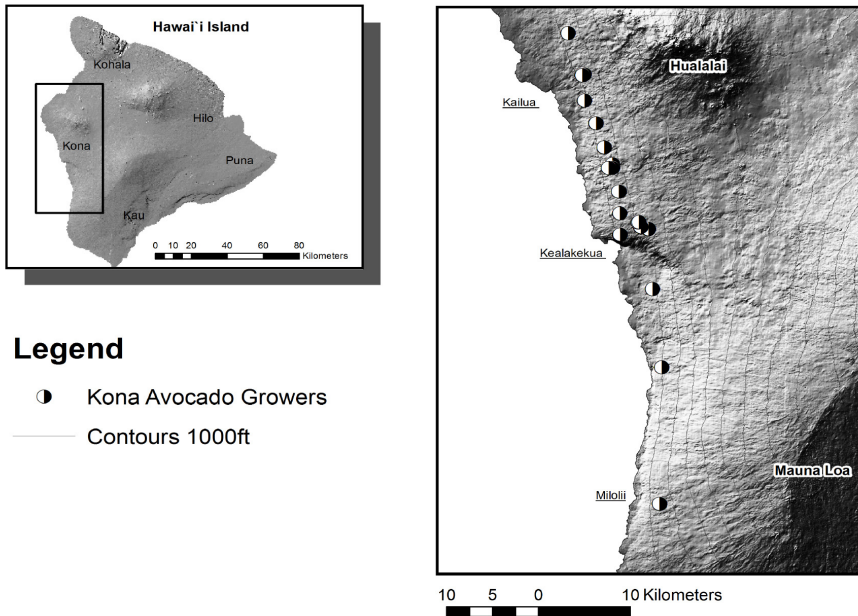


Figure 1. Contour map of the distribution of the first 16 avocado farms surveyed.

America Inc., Keokuk, IA) buffered with 4% borax (U. S. Borax, Inc., Scottsdale, AZ) in water. Approximately 30% polypropylene glycol (Better World Manufacturing, Fresno, CA) was added to this mixture at time of deployment.

The traps were deployed in or near the avocado trees and were separated by a distance of 3 meters or more. These were monitored bi-weekly. By Sept 6, 2006 traps had been deployed at 14 farms (18 trapping sites). These selections were based on accessibility, acreage of more than 1 acre of avocado in production and geographic location along the Kona altitudinal gradient from 426 m to a 914 m. The full complement of 18 farms (22 sites) (Fig. 1) had been deployed by 13 December 2006. These were continuously monitored until 3 October 2007.

The male-specific lures used are known to have a different level of attractiveness for their particular target species. Generally methyl eugenol (ME) has the longest range of attraction, cue-lure (CL) less so, and trimedlure (TM) has the least range of attraction (Jang and Light 1996).

Protein bait traps generally have a more consistent level of attractiveness over the three fly species, although there are some species differences (Barry et al. 2006, Heath et al. 1994, Vargas and Prokopy 2006). Protein bait traps also have a short range of attraction, and thus are a better measure of the population of flies in the trapping site (McQuate and Vargas 2007). They also capture females, which is the important sex to monitor, because they oviposit in the fruit.

Infestation data. The sampled orchards were spread along the Kona District at elevations between ~250m and ~800m. (see Fig. 1 for elevation contours), and, therefore, avocado fruit ripened at different times. Fruit collections began on 8 June 2006. Collections were small at first, until 9 August 2006 when 35 fruit were collected from 3 farms. Sampling of between 30 to 40 fruit ca. every 2 wk continued. These were taken from 3 different orchards (rotating

between the 20 sites) on each collection date. The availability of ground fruit exhibiting signs of damage varied greatly, and consequently the number of fruits collected at each farm also varied greatly. Sampling was terminated for logistical reasons on 22 May 2007, at which time 519 fruit had been collected out of 616 fruits examined. All fruit collected had some indication of damage, whereas unblemished fruit with pedicel attached was examined but not collected. The first 51 fruits collected were not evaluated for type damage, and all but 21 of the fruit were rated as being mature or immature. Thereafter, fruits were collected from the ground and categorized as "missing pedicel," "firm epidermis," "epidermis slightly soft to pressure," "scarred epidermis," "black epidermis near pedicel," "black spotting on epidermis," and "epidermis all blackened." They were then immediately sealed in plastic bags. At the Pacific Basin Agricultural Research Center, Hilo HI, the fruit was weighed and transferred to rearing buckets which consisted of two 1 liter polyethylene buckets (408-30N, Highland Plastics, Mira Loma, CA) with 4 drain holes to allow liquid from the decomposing fruit to drain. The inner bucket was filled with sand to a depth of 2 to 4 cm. Each fruit was suspended on a platform formed from hardware wire screen of 15 x 15 cm mesh. Each bucket contained one fruit and was sealed with a lid containing a screened window 13 cm in diameter and covered with 20 x 20 mesh Lumite® screen (Synthetic Industries, Gainesville, GA). Buckets were held at ambient temperature inside a 2-story well ventilated warehouse for 2 to 4 wk. The larvae that pupated in the sand were sieved after 1 and 2 weeks. Puparia were collected in polyethylene cups (GS-309-06, Highland Plastics, Mira Loma, CA) (11.5 cm diameter by 7.5 cm depth) and covered with organza cloth (a nylon fabric with fine weave similar to cotton organdy) and held at ambient room temperature until emergence of fruit fly and parasitoid adults was complete. Puparia and emerged adult fruit flies as well as parasitoids were counted and sexed.

Trapping data were analyzed using SAS Proc. SUMMARY and GLM (SAS Institute, 1999, Cary, NC), to derive all combination of means by orchard, date, and lure. Emergence data were analyzed by SAS Proc. FREQ (SAS Institute, 1999, Cary, NC). Trend curves were computed for seasonal data collected on various dates and on many small farms using best fit regression analysis (SAS 1999). For proportions in fruit quality stages, binomial statistics (Lowery 1998–2007) were used to evaluate the significance of the low infestation found against similar analyses of a larger data set by Liquido et al. (1995).

Results and Discussion

Adult fly populations. Over the 15 months of monitoring in Kona avocado orchards, the abundance and distribution are reported for *B. dorsalis*, *B. cucurbitae*, and *C. capitata*. In avocado orchards in Kona *B. dorsalis* captures in ME traps were consistently highest (mean 49.11 FTD at maximum), *C. capitata* captures in TM traps were second highest, and actually exceeded *B. dorsalis* captures on 1 November 2006 (Fig. 2), and again from 5 September 2007 until trapping ended. *B. cucurbitae* captures in CL traps were consistently the lowest throughout the 15 month survey.

The pattern of female fly captures in protein bait traps over the 15 months indicated a different relative abundance of flies in the avocado orchards (Fig. 3) than one would interpret from male lure trap capture data. The greatest difference between *C. capitata* and *B. dorsalis* occurred in the month of October when ripe coffee was abundant (Vargas et al. 2001). At that time *C. capitata* reached a peak mean of 2.93 fly/trap/day (FTD). Coffee is an important host for *C. capitata*, which largely explains why *C. capitata* is abundant in the Kona District (Liquido et al. 1995; Vargas et al. 2001), whereas it is very rarely found in eastern Hawaii island at low elevation (unpublished survey of Puna district in 2000–2001). December is the only time that captures of female *B. dorsalis* exceeded 1 FTD. Female

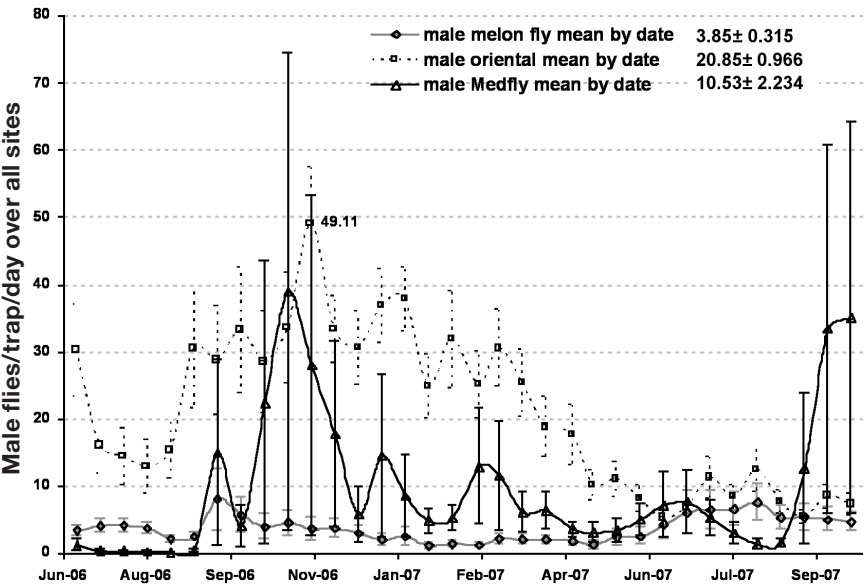


Figure 2. Biweekly mean male FTD per species captures with the appropriate lure. Mean and SEM by species over all the dates is also included.

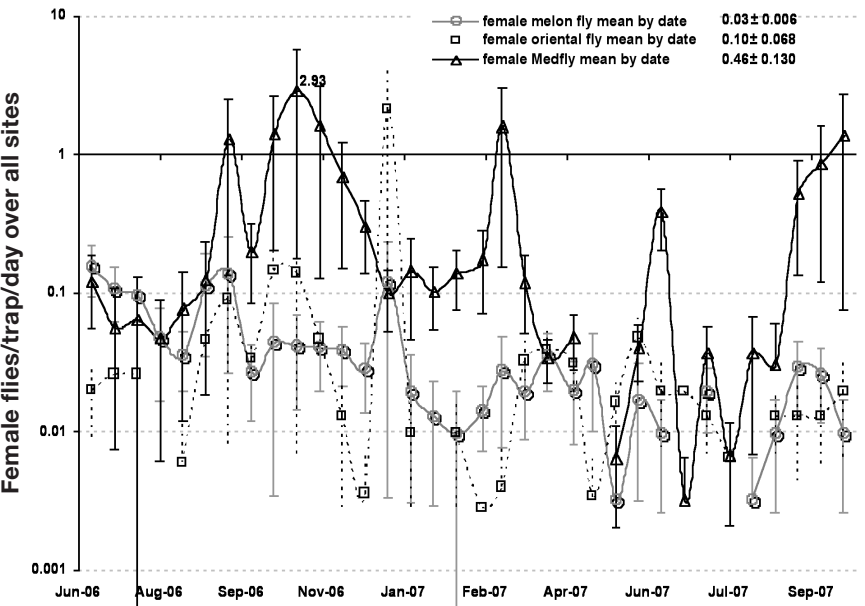


Figure 3. Biweekly mean female FTD (on a logarithmic scale) per species captured in protein bait traps. Means and SEM by species over all the dates is also included.

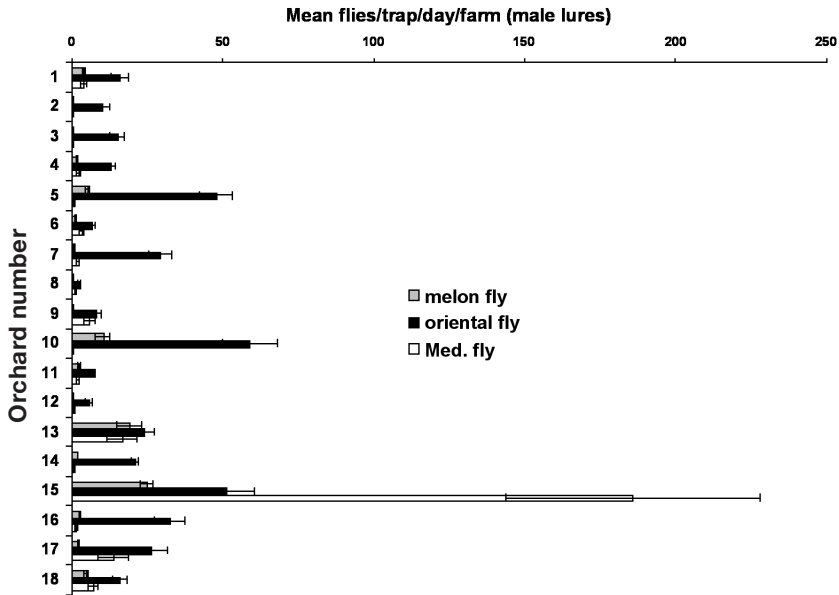


Figure 4. Farm means of the three species of FTD and SEM over the entire period of collection using methyl eugenol for oriental fruit fly, cue-lure for melon fly, and trimedlure for *C. capitata*.

C. capitata captures in the avocado orchards were consistently higher than the other two species, and exceeded 1 FTD throughout most of the coffee ripening period and thereafter (20 September to 13 December 2006). For the greater part of the year, captures of female *C. capitata* also remained lower than 1 female FTD. The *C. capitata* females again exceeded 1 FTD in September of 2007.

The forgoing calculations were averaged over all the farms that were surveyed in the North and South Kona Districts. However, within-farm means revealed species differences at the different farms (Fig 4). Whereas *B. dorsalis* was distributed throughout the farms, *C. capitata* was highly concentrated in one farm (Farm 15), and relatively few flies were caught on average at the other farms. Farm 15 also had high populations of melon flies, although it is unlikely that the *B. cucurbitae* is attracted to the avocado fruit (Fig. 4). Wild hosts of *B. cucurbitae* are common in the Kona District (Vargas et al. 2004).

A closer examination of the variation in *C. capitata* captures in protein bait indicates that female captures averaged <1 FTD at most farms fly/trap/day (Fig. 5).

The great variation in mean annual fly captures between the individual farms, raises the question of how consistent those differences are throughout the year. Plotting the bi-weekly captures for all farms at different elevations, it is apparent that there is high variation in local fly captures due to the presence of many different fruits, other than avocado, in the vicinity of the avocado farms. Many tropical fruits are cultivated in the Kona District, and mango, guava and other fruit species grow wild. Analysis of variance over all species caught in protein bait traps indicate that farms were the most significant source of variation ($F=14.88$; $df=17, 50$; $P<0.0001$), although only Farm 15 was significantly higher than other farms. Date was less significant ($F=1.49$; $df=33,50$; $P<0.0365$). Similarly, over all species

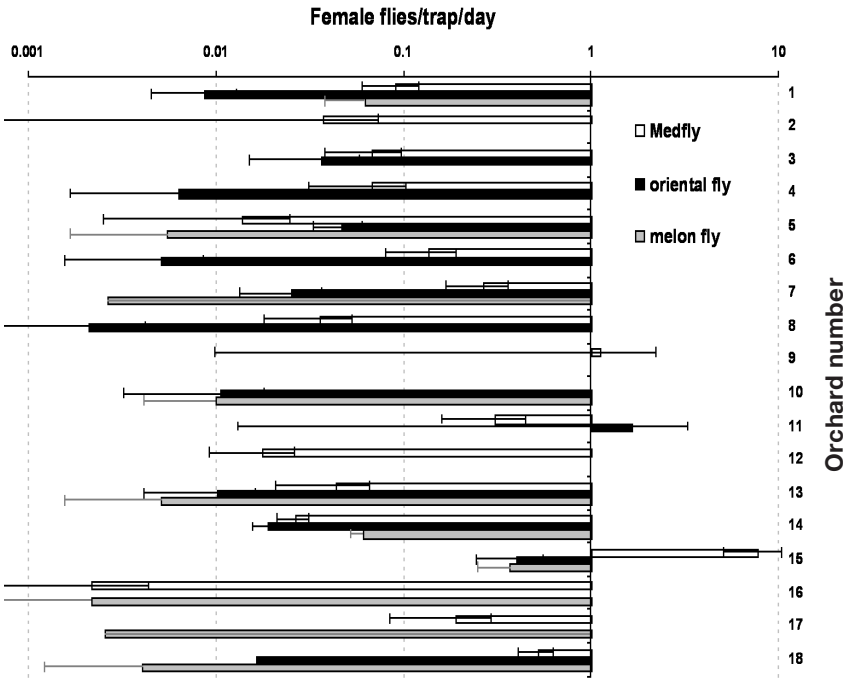


Figure 5. Farm means and SEM of female flies by species captured per sampling date in protein bait over the period of collection.

caught in male lure traps, the farms ($F = 28.87$; $df = 17, 50$; $P < 0.0001$) were more variable than the dates ($F = 1.81$; $df = 33, 50$; $P < 0.0032$). Only fly captures at Farm 15 differed significantly from all other farms, although, Farm 10 and Farm 13 had among the highest mean capture rates for lure traps.

Mean male *B. dorsalis* captures at the different farms ranged between 0.2 and 15.4 FTD. In Fig. 6 A, a best-fit polynomial over all orchards was associated with only 19.9% of the total variation ($F = 22.16$; $df = 6, 534$; $P < 0.0001$) reflecting the large differences in captures between orchards. Seasonal variation is illustrated in Fig. 2, where a best-fit high-order polynomial correlation to mean FTD was $R^2 = 0.9095$; $F = 13.07$; $df = 14, 19$; $P < 0.0001$. *B. dorsalis* was the only species that exhibited a linear decline in mean male population over time ($R^2 = 0.3667$; $F = 18.53$; $df = 1, 32$; $P < 0.0001$). The 36.7% of variation associated with this linear model may reflect a decline to a low population in September. However, because the population was higher in September of 2006, the linear decline more likely reflected the localized suppression effect of male annihilation in the area of the traps due to the highly attractive methyl eugenol.

The range of *C. capitata* was the highest (range: 0 to 786.3 FTD, Fig. 6 B), which one might expect to be highly correlated to the amount of coffee grown in the vicinity of the avocado farms. In general, the variation in captures in orchards was so great that a best fit 6th order polynomial model over data from all orchards was associated with only 2.11% of the total variation in *C. capitata* FTD ($F = 2.90$; $df = 4, 538$; $P = 0.0213$). However, the best-fit higher order polynomial correlation to mean FTD to date was $R^2 = 0.8741$; $F = 8.33$; df

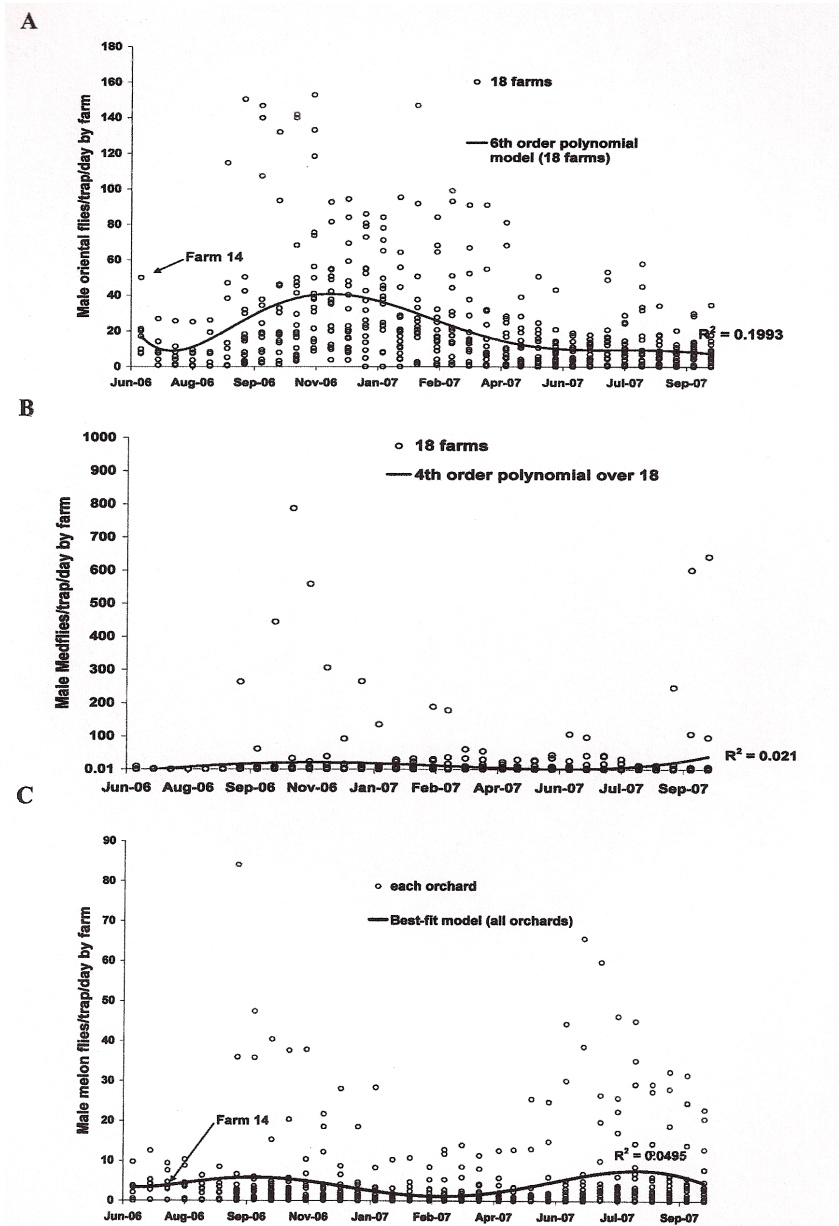


Figure 6. A. Best fit regression of *B. dorsalis* FTD over all farms by date. Farm 14 was the only farm with replicate samples from which a mean and SEM by date could be derived (grey error bar). B. Best fit regression of male *C. capitata* FTD over all farms by date. In order to show the great variation, data are plotted on a log scale (this required adding 0.001 to every observation to eliminate zeros). C. Best fit regression of male *B. cucurbitae* FTD over all farms by date.

Table 1. Infestation results of ground fruit collections at 14 of the 22 farms where traps were deployed.

Farm	No. Fruit	Mean flies/g	SEM
Overall			
sampled farms	489	0.000013	0.000009
2	45	0.000000	0.000000
3	1	0.000000	-
4	47	0.000000	0.000000
5	12	0.000542	0.000366
6	12	0.000000	0.000000
8	110	0.000000	0.000000
16	104	0.000000	0.000000
10	10	0.000000	0.000000
13	10	0.000000	0.000000
14	4	0.000000	0.000000
15	15	0.000000	0.000000
17	10	0.000000	0.000000
18	108	0.000000	0.000000

=15,18; P = 0.0010, (Fig. 2), reflecting significant seasonal variation .

B. cucurbitae (Fig. 6 C.) presented an unusual case. Sixteen of the traps caught <15 FTD, except for those in orchards at Farms 13 and 15. These traps cycled from > 40 to < 10 FTD, suggesting there is high variability in melon fly numbers in individual avocado orchards. A best-fit polynomial model was associated with 4.62% of the total variation between orchards (F = 6.53; df = 4,539; P <0.0001). The best-fit 10th degree polynomial correlation of the mean male melon FTD of all farms to date was R² = 0.8078; F =9.67; df = 10, 23; P <0.0001 (Fig. 2).

What are the implications of these data for the probability of fruit fly infestation of avocado? Because male lure trap captures varied with locality and season and attracted flies from large distances, they are probably of limited value in predicting numbers of fruit flies within small avocado orchards. On the other hand protein bait captures, because they captured females and attracted flies from short distances, were a better indication of female flies found within orchards. The actual implication for fruit infestation can only be determined from fruit samples.

Fruit infestation. Collecting damaged fruits from the ground, maximized the likelihood of finding infested fruit. Nevertheless, it was not until 1 February 2007 that 4 larvae were found in 2 of the fruits collected. Each fruit contained 1 male and 1 female *B. dorsalis*. Both infested fruits were found on Farm 5 (Table 1). This isolated infestation by *B. dorsalis* represents only 0.38% of the total fruit sampled. A total of 519 fruit were held to determine infestation, and no additional larvae were found.

The condition of the fruit is described in Table 2, where the percentage of fruit within seven categories is summarized. Only 20.2% of all the fruit collected was mature (i.e. fully ripened) but 98.8% of the sampled fruit had lost the pedicel, which is a region of the fruit more likely to be attacked by females (Liquido et al. 1995). Percentage in other damage categories is listed in Table 2. Any of the categories of damage are likely points for the flies to lay their eggs.

Table 2. Condition of the fruits observed and collected not including unblemished fruit with pedicel.

Category of fruit by condition of epidermis	Number of fruit observed	Percent in the category	Lower Exact Conf. Limit	Upper Exact Conf. Limit
Missing pedicel	468 ¹	98.08	-1.04	+1.70
Firm		28.63	-4.33	+4.05
Slightly soft		70.94	-4.08	+4.34
Scarred		52.56	-4.61	+4.63
Black pedicel end		13.68	-3.44	+2.99
Black spotting		45.73	-4.63	+4.58
All blackened		8.55	-2.91	+2.37
Mature fruit	495 ²	20.2	-3.81	+3.45

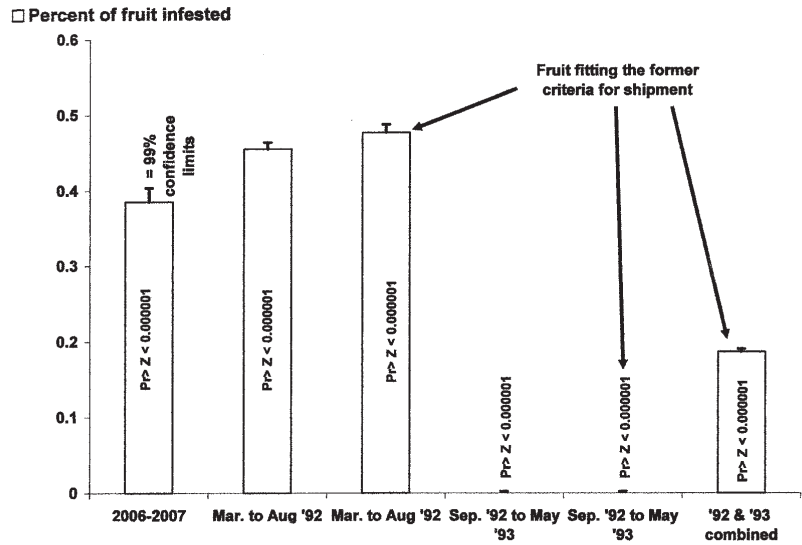
¹ Only 51 of the 519 fruit collected were not categorized.

² Of the 519 fruit, 495 were mature fruit.

A study of avocado infestation was conducted by Liquido et al. (1995) in the spring and summer of 1992. The sample was much larger than the 2006–2007 survey and was conducted in a year when weather fluctuations caused anomalous ripening (i.e., an early warm spell was followed by a cold spell that stopped the normal progression of ripening). Under these conditions, they found that most infestation occurred in fruit that was either punctured, had lost its pedicel, or was firm ripe but spongy and without a pedicel. The proportion of the fruits in these categories that were infested was <0.5%. A second trial by Liquido et al. (1995) was conducted between January and March 1993. These results are divided into infestation of the fruit that met the USDA-APHIS’s criteria to allow export and those that did not. They are presented as the percentage of fruit that were infested (Fig. 7 A) and a percent of larvae and pupae found in the fruit (Fig. 7 B). The binomial confidence level for both graphs are calculated at the 99% probability level and $Pr.> Z < 0.000001$ in each proportion. Because Liquido et al. (1995) reported that infestation occurred only in the 1992 sample, the mean over the two sampling periods is included in the graph.

In comparing the 2006–2007 fruit sampling to the two sampling periods in 1992 and 1993, it should be noted that 97.9% of the 2006–2007 ground fruit sample was without pedicel, only 73.2% of the fruit had firm epidermis, and the majority had some sort of damage or were in later stages of ripeness. These would have been more likely to be infested than intact fruit in trees. Yet the proportion of fruit infested was 0.385%, only slightly less than the 1992 sample. However, the percent of larvae per fruit (0.771%, computed from data in Fig.12 B from Liquido et al., 1995) was 87.5% less than the sample of 3,297 fruits (6.157%) collected by Liquido et al. in 1992. Applying the binomial confidence intervals indicates significant differences between the 2006–2007 samples and the fruit with pedicel collected in 1992. Even when re-calculating the results of Liquido et al. based on their total sample of fruit (8,036) that met the export criteria (i.e. fruit including all unblemished fruit picked on the tree with pedicel attached), the resulting proportions differ significantly at the 99% confidence interval. Note that the current, much smaller sample of ground fruit gave a higher percent of fruit that were infested but a lower percent of larvae per fruit than the tree-harvested fruit of Liquido et al. (1995). Infestation of avocado by fruit fly is possible, but is likely to occur in damaged fruit or fruit that has lost its pedicel.

A.



B.

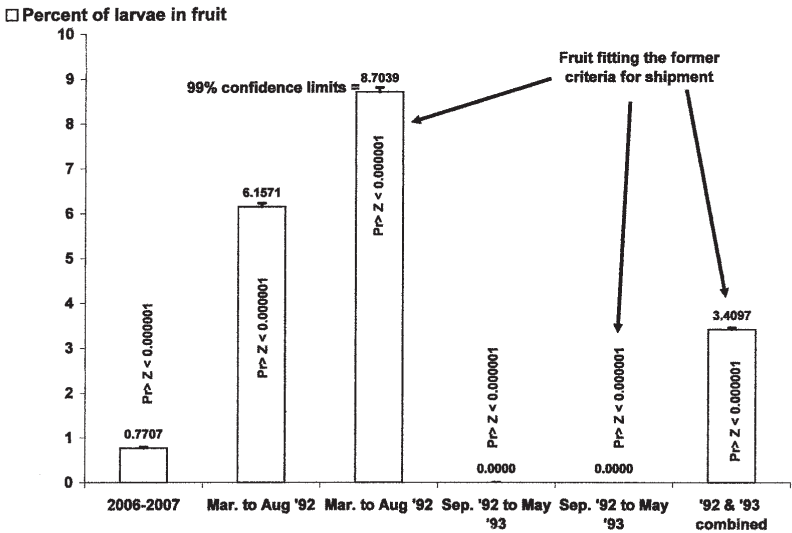


Figure 7. A. Percent of fruits that were infested from the current collection compared to the collections of Liquido et al. (1995). The latter is calculated for all fruit, and for only the fruit which met the criteria for shipment at that time. B. Percent of larvae in fruit (larvae/fruit) from the current collection compared to the collections of Liquido et al. (1995). The latter is calculated as in 7A.

Conclusions. In this study, the probability of infestation was very low, but not excluded entirely, in spite of the relatively high numbers of *B. dorsalis* and *C. capitata* found in some orchards. The current study confirms the results of the more thorough study in 1992–1993. Liquido et al. (1995) noted that between September 1992 and May 1993 “not a single fruit sampled had *B. dorsalis* infestation” in spite of relatively consistent numbers of *B. dorsalis* captured in the orchards throughout the experiment. Our study supports the conclusions of those authors that variations in the weather conditions can affect the susceptibility of avocado to fruit fly infestation. Under “normal” weather conditions, avocado has very little susceptibility to fruit flies (Armstrong 1991), but any kind of damage induced by weather, birds, mice, cracking or bruising can increase the susceptibility.

Although the 2006–2007 fruit sample from avocado was all ground fruit (reared out individually), fruit infestation was low. Given that this fruit mostly had damage that would encourage fruit flies to lay eggs, and the fact that only two fruits were infested, out of 519 sampled, supports the conclusion that, under field conditions, avocado is only marginally susceptible to attack by *B. dorsalis*, and less so to the other species.

Liquido et al. (1995) did not distinguish between male and female captures, so the current study gives a measure of the number of female fruit flies present in avocado orchards. The combination of low susceptibility of the damaged and blemished fruit and the relatively low captures of female flies in the orchards should be considered in evaluating the advisability of allowing Hawaiian avocados to be exported.

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